control at all time points (p<.001). When given individually, these biologic agents had less pronounced effect on the preservation of Safranin O staining, yet remained better than other tested agents.
Conclusions: PTOA therapies will be most effective if a multi-mechanistic approach is utilized to prevent chondrocyte death and matrix loss and to stimulate reparative responses. Combination of treatments that target three distinct mechanisms: cell membrane protection (P-188), inhibition of catabolism (anti-TNF-α), and stimulation of anabolic responses (OP-1) appears to be the most promising approach to biologic intervention of PTOA. Importantly, this Triad shows promise for rapid translation into clinical settings since all three factors have already been approved by the FDA for other use.

83
INTRA-ARTICULAR BLOOD COAGULATION AGGRAVATES JOINT DAMAGE AFTER A BLEED IN A CANINE *IN VIVO* MODEL

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Purpose: Joint bleeding due to trauma, major joint surgery, or hemophilia leads to joint damage. However, it is unclear if there are differences between coagulating blood and anticoagulated blood with respect to joint degeneration, especially *in vivo*. Therefore, we evaluated in a canine *in vivo* model whether intra-articular blood exposure is more destructive in case of coagulating blood compared to anticoagulated blood, and whether inflammation plays a role in the cartilage damaging process.
Methods: In 7 dogs left knees were injected with coagulating blood 4 times a week in week 1 and 4; right knees with saline. In 7 other dogs anticoagulated, heparinized blood was injected with heparinized saline as control. Ten weeks after the last injection cartilage matrix turnover and synovial inflammation were analyzed *ex vivo*. To study cartilage damage without involvement of synovial inflammation, healthy full thickness human cartilage explants were exposed *in vitro* to coagulating and anticoagulated blood for 4 days (n=6). Cartilage matrix turnover was determined at day 16.
Results: Canine knees injected with coagulating blood showed an increase of both newly formed and total (resident) glycosaminoglycans of 9% and 15%, respectively (p=0.04 and p=0.01, respectively), as well as a decreased proteoglycan content of 6%. Injection with coagulating and anticoagulated blood caused an increase of proteoglycan synthesis rate of 24% and 14%, respectively (p=0.01 and p=0.04, respectively), as a characteristic of (ineffective) repair activity. Intra-articular injections with coagulating blood led to more synovial inflammation on macroscopy as well as histology (p<0.001and p=0.01, respectively), in contrast to knees injected with anticoagulated blood. Coagulation of blood *in vitro* resulted in more cartilage damage compared to anticoagulated blood. This was expressed as more reduction of proteoglycan synthesis rate by coagulating blood compared to anticoagulated blood (17% difference; p=0.05), and a higher release of proteoglycans after exposure to coagulating blood compared to anticoagulated blood (94% difference; p=0.01).
Conclusions: This study shows that coagulating blood causes more long-lasting *in vivo* joint damage than anticoagulated blood; directly on cartilage and via inflammation. In case of joint surgery prolonged anticoagulation might limit the harmful effects of intra-articular blood. Moreover, aspiration of blood would prevent joint damage.

84
DEPLETION OF PERLECAN DOMAIN I HEPARAN SULFATE REGULATES FIBROBLAST GROWTH FACTOR ACTIVITY IN CARTILAGE AND PROTECTS AGAINST CARTILAGE LOSS AND OSTEOPHYTE DEVELOPMENT IN POST-TRAUMATIC OSTEOARTHRITIS

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Purpose: Fibroblast growth factors (FGF) -2 and -18 have been suggested as potential therapeutic agents in osteoarthritis (OA). FGF-18 promotes chondrogenesis and cartilage repair in vitro and in animal models. FGF-2 has demonstrated both chondroprotective and pro-degradatory properties, potentially dependent upon variable receptor expression. Both FGF-2 and -18 signalling require heparan sulfate (HS) as a coreceptor. Given its pericellular localisation, perlecan (HSPG2) has been implicated as a critical agent in FGF-2/18 activities. We directly investigated the role of perlecan domain I HS in the onset and progression of post-traumatic OA, and whether the effects can be attributed to altered FGF-2/18 signaling in articular cartilage.
Methods: Male 10-weeks old wildtype (WT) and Hspg2 exon-3 null (in which the domain I HS chains are ablated) mice underwent unilateral destabilisation of the medial meniscus (DMM). Cartilage damage, subchondral bone sclerosis and osteophyte development were scored 4- and 8-weeks post-operatively. Femoral head cartilage (3 week old mice both genotypes) were cultured ± IL-1 (10ng/ml) ± FGF-2 or -18 (0, 1, 10, 100ng/ml) for 4 days. Sulfated glycosaminoglycan (sGAG) released into the media was quantified. Cartilage explant gene expression was analysed by real time PCR.
Results: There were no differences in cartilage aggrecan loss or structural damage 4 weeks post-DMM surgery between genotypes. At 8 weeks exon-3 null mice had significantly less cartilage erosion than WT (Fig 1A). The increase in subchondral bone sclerosis post DMM did not differ between genotypes, but osteophyte size was significantly reduced in exon-3 null mice at 8 weeks (Fig 1B).
There were no genotypic differences in either basal- or IL-1-stimulated sGAG release from cartilage explants. FGF-2 did not alter sGAG release ± IL-1 in either genotype at any dose. FGF-18 alone did not alter sGAG release in either genotype, but significantly attenuated IL-1-induced sGAG loss (~35%) only in exon-3 null cartilage.
There were no differences between genotype in gene expression in control or IL-1 cultures. FGF-2 alone was generally "pro-catabolic", up-regulating Adamts4 & 5 and Mmp13 mRNA in exon-3 null cartilage. In the presence of IL-1, FGF-2 decreased WT RNA yield and Gapdh mRNA, suggestive of the detrimental effect of FGF-2 in injured cartilage. FGF-18 in the absence of IL-1 decreased Adamts4 in both genotypes, and markedly suppressed Mmp2 expression only in exon-3 null cartilage. Unlike FGF-2, FGF-18 did not affect cell viability in either genotype in the presence of IL-1.
Figure 1. Cartilage erosion (A) and osteophyte size (B) in surgically induced OA in WT and Hspg2 exon-3 null mice (n = 17-20/group).
Conclusion: Increased mRNA expression in response to FGF-2 (Adamts4/5, Mmp13) and FGF-18 (Mmp2) in exon-3 null cartilage confirmed the importance of perlecan domain I HS, suggesting it acts by sequestration and reduced presentation to receptors in cartilage. The in vitro data suggests that reduced cartilage erosion in exon-3 null mice post DMM surgery may be associated with increased FGF-18 rather than altered FGF-2 signalling in chondrocytes in the absence of perlecan domain I HS. The mechanism behind reduced osteophyte size in exon-3 null mice is unclear but may result from altered stem cell differentiation, endochondral ossification, and/or activities of growth factors (e.g. TGFβ) implicated in osteophyte development. We suggest that the lack of perlecan domain I HS chains negates FGF-18 sequestration, altering signalling effects and in vivo cartilage remodeling. Controlling the synthesis and/or turnover of chondrocyte perlecan and/or HS substitution may provide a therapeutic target alone or in combination with FGF-18 in the treatment in OA.